Chronic Changes in Inputs to Dorsal Y Neurons Accompany VOR Motor Learning

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Chronic changes in inputs to dorsal Y neurons accompany VOR motor learning. J Neurophysiol 95: 1812–1825, 2006. First published November 30, 2005; doi:10.1152/jn.01061.2005. Gain changes in the vestibulocular reflex (VOR) during visual-vestibular mismatch stimulation serve as a model system for motor learning. The cerebellar flocculus and its target neurons in the brain stem (FTN) are candidates for the storage of these novel VOR gains. We have recently studied the changes in vertical flocculus Purkinje cells after chronic VOR motor learning. Recently we recorded Y neurons (a vertical type of FTNs) after chronic VOR motor learning and compared these records with vertical floccular Purkinje cells to document any changes in inputs to FTNs and understand how Y neurons and the vertical Purkinje cells fit into a general model for the vertical VOR. Analysis illustrates that the changes observed in Purkinje cells are not transferred to Y neurons, suggesting that the gain of their synaptic interconnection was modified. We quantified changes in both populations and employed simulations to study changes in parallel pathways to FTNs and to extract the role of the flocculus in VOR adaptation. Low-gain adaptation results in more drastic changes than its high-gain counterpart, causing increases in head velocity sensitivity in parallel pathways. Simulations suggest that cerebellar and brain stem plasticity both participate in novel VOR gain storage and that results obtained following floccular lesion are the product of different mechanisms than those operating in the intact animal.

INTRODUCTION

The vestibulocular reflex (VOR) has long been used as a model system to elucidate neural correlates of learning and memory. VOR gain is measured during head rotation in the dark as the ratio between eye and head velocity. Gain in the naïve animal is close to 1 (Miles and Eighmy 1980; Paige 1983) but can be increased or decreased by training with conflicting visual-vestibular stimulation (Gonshor and Melvill Jones 1973).

Basic VOR neuronal circuitry consists of three pathways. Vestibular information is conveyed indirectly to the cerebellar flocculus in one and directly to brain stem neurons in the second. Some of these brain stem cells in the second pathway receive input from the flocculus and are therefore called flocculus target neurons (FTNs). The loop through the flocculus carries both eye- and head-velocity information and transmits this to the FTNs. The third, parallel processing pathway does not involve the flocculus and consists of position-vestibular-pause (PVP) cells. Previous studies suggested that the flocculus plays an important role in the acquisition but not the retention of new VOR gains (Miles and Lisberger 1981). Broussard and Kassardjian (2004) showed that acute VOR motor memories are lost after flocculectomy, but chronic memories are largely retained. The authors suggest that the cerebellum has a major role in memory retention in the acutely adapted animal and that memories are shared between cerebellum and brain stem in the chronically adapted animal (cf. also Kassardjian et al. 2005; Nagao and Kitazawa 2003; Pastor et al. 1994). However, flocculectomy can be a misleading paradigm because it modifies the circuitry; specifically, transforming the circuitry from a combined feed-forward and -back system to a solely feed-forward system. This caveat has been overlooked and might be one reason why a role for the cerebellum in memory storage remains controversial (Ito 2002).

It is tacitly assumed that firing patterns of neurons within VOR circuitry might reflect the storage of novel VOR gains. Recordings suggest that chronic VOR motor learning is the result of brain stem and cerebellar plasticity. Thus Lisberger et al. (1994a) showed that floccular Purkinje cells change their head-velocity sensitivity monotonically after chronic VOR adaptation simultaneous with a change in the head-velocity sensitivity of FTNs, suggesting that both the flocculus and the brain stem participate in VOR gain storage (Lisberger 1994). Blazquez et al. (2003) added that both head- and eye-velocity sensitivities of flocculus Purkinje cells change after VOR adaptation.

Model simulations have improved our understanding of VOR motor learning in the horizontal VOR (Lisberger 1994); however, this has not yet been pursued for the vertical VOR. Vertical Purkinje cell sensitivities to eye, head, and retinal slip parameters have been fully characterized before and after VOR adaptation (Blazquez et al. 2003). Partsalis et al. (1995a,b) offered compelling evidence for changes in vertical FTNs located in the dorsal Y group nucleus (Y neurons), but quantification of sensitivities to eye and head parameters were not fully evaluated. Furthermore, the vertical counterpart of the horizontal third pathway or PVP cells has not yet been studied.

Recently, we recorded the activity of Y neurons before and after chronic VOR motor learning. Y neurons have upward eye- and upward head-velocity signals (Chubb and Fuchs 1982). The only known source of eye velocity to Y neurons arrives from the flocculus (Partsalis et al. 1995b; Rambold et al. 2002). The head signal, however, arrives to Y neurons via two pathways: the flocculus, which sends an upward head velocity signal (inhibitory synapses convert the downward...
head velocity of Purkinje cells to upward head velocity) (Langer et al. 1985; Partsalis et al. 1995b), and interneurons with either up or down head-velocity signal are located in the superior vestibular nucleus (SVN) (Blazquez et al. 2000; Sato and Kawasaki 1987). In the normal gain or naive animal, Y neuron head-velocity sensitivity arrives almost exclusively from the flocculus because inputs from up-head and down-head velocity interneurons in the superior vestibular nucleus cancel each other at the level of the dorsal Y group, as suggested by pharmacological inactivation of the cerebellar flocculus (Partsalis et al. 1995b).

This study has two major aims: to study the changes in the pathways carrying information to Y neurons after chronic VOR motor learning and to model the neuronal substrate responsible for the vertical VOR in the normal and chronically adapted animal. Thus we first quantify the changes in neuronal sensitivity to head and eye parameters in Y neurons after chronic VOR motor learning and compare these changes with those observed in vertical Purkinje cells in the flocculus. Second, we estimate the changes in the third pathway that reflect the adapted state. Third, we study the role of cerebellar plasticity in the retention of new VOR gains using simulations that do not disrupt the feedback loop. Results show that synaptic plasticity is likely to occur in several elements of the circuitry and in multiple pathways, namely Purkinje cells and Y neurons; the signal transfer from Purkinje cells to Y neurons is under regulatory control after VOR motor learning; and simulations replicate the results obtained in flocculotomized animals. We conclude that changes at a single site are not sufficient to account for the learned behavior and most importantly that behavior in lesioned animals results from different mechanisms than those observed in the intact animal.

METHODS

Animal preparation and recording setup

Two squirrel monkeys (monkeys 062 and 065), weighing 800–1,000 g, were used to record the neuronal activity of neurons in the dorsal Y group. In a first surgical procedure, we implanted a head post for head fixation and a single eye coil in the right eye to monitor vertical and horizontal eye position. After a recovery period of 3 wk (065) or several months (062), animals were implanted with a chamber for electrophysiological recordings. The chamber was implanted stereotaxically with its center aimed at the ipsilateral superior vestibular nucleus (SVN; 2.0 posterior, 0.7 lateral and 15° tilted medially–laterally). Amphetamine sulfate (0.15 ml/kg po) was given while recording to maintain constant alertness. Experimental protocols were approved by the Washington University Committee on Animal Care and performed in accordance with National Institutes of Health guidelines.

During the recording sessions, animals were seated in a primate chair and placed atop a rotating table. For visual stimulation, we used an optokinetic system consisting of a transparent drum with black strips. A light source located in the center of the drum projected the image of the stripes onto a white cylindrical screen placed in front of the animal. The axis of rotation of the rotating chair and drum coincided and were perpendicular to the earth plane. To induce vertical vestibular stimulation and vertical optokinetic stimulation (OKS), we tilted the animal onto its right side with the axis of rotation passing through its interaural axis, thus minimizing translational effects caused by the otolith organs. To induce VOR suppression (VORS), we moved the optokinetic system and the chair together, and to induce VOR enhancement (VORE), we moved the chair and the drum at the same frequency and velocity but in the opposite direction.

VOR in the dark (VORd) was evoked by rotating the animal in a dark room. The gain of the eye coil system was calibrated during passive head rotations in the light (0.5 Hz and 40°/s) in the normal gain state.

Behavioral training

Animals were chronically trained to either increase or decrease their VOR gain by the wearing of minimizing (×0.5) or magnifying (×2.2) goggles for several weeks to months. Goggles were custom made and built to fit each individual animal. Animals were observed to behave normally in their home cages.

Recording procedures

Horizontal and vertical eye position, head velocity, drum velocity, and neuronal activity were acquired using a Power 1401 (CED) and Spike 2 software. Neuronal activity was simultaneously recorded as a waveform sampled at 40 kHz and events using an on-line window discriminator.

Before searching for Y group neuron, we constructed a map of the areas surrounding the ipsilateral vestibular nucleus, including the ipsilateral abducens, anterior and posterior SVN, dentate and dorsal Y group nuclei. Y neurons were identified by their location, and their typical response during OKS (Partsalis et al. 1995a). Once a Y neuron was identified (using an OKS paradigm), we recorded activity during OKS (30 cycles, 0.5 Hz, and 40°/s), spontaneous eye movements in the light, head rotation in the dark (30 cycles, 0.5 Hz, and 40°/s) spontaneous eye movements in the dark, and VOR suppression and/or VOR enhancement (see Blazquez et al. 2003 and Parsalis et al. 1995a).

Analysis method

The analysis methods have been previously described (Blazquez et al. 2003; Hirata and Highstein 2001). Data were exported off-line into Matlab (The MathWorks) for analysis. Only smooth eye movements and neuronal response were considered. Saccades were first removed automatically by setting an acceleration threshold (200–400°/s) and later confirmed by visual examination (manual desaccading was sometimes necessary). Two analysis methods were employed to account for the neuronal firing rate during visual-vestibular stimulation. In a first analysis, eye, head, and neuronal data were averaged over cycles of sinusoidal rotation and the result approximated by a sinusoidal fitting. This provides an estimate of the overall neuronal modulation and eye movement and was described in terms of gain (VOR gain and neuronal gain) and phase (Partsalis et al. 1995a).

A second analysis method was used to obtain the neuronal sensitivities to eye, head, and retinal slip parameters. This method is similar to that previously used to extract information from Purkinje cells firing rate (Blazquez et al. 2003; Hirata and Highstein 2001), and consists of fitting the raw (nonaveraged) neuronal response using a multiple linear regression model that includes raw eye, head, and retinal slip information (Hirata and Highstein 2001). Thus, this method models the firing rate of Y neurons using eye, head, and retinal slip information. The validity of the model is supported by the existence of these signals in the floccular Purkinje cells (input cells to the dorsal Y group) and the existence of a head-velocity input to the Y neurons of nonfloccular origin (Blazquez et al. 2000). Similarly to the horizontal VOR we assume that all signals add together at the Y neuron level (Lisberger 1994). The order of parameter extraction from the neuronal firing rate is identical to that used previously for Purkinje cells (Blazquez et al. 2003; Hirata and Highstein 2001).

In short, the neuronal and behavioral response during OKS was used to extract the retinal slip and the eye-related components (Eq. 1). We could extract retinal slip and eye-movement information from OKS because eye movements during each cycle of OKS were not identical; however, in those cases where the variance inflation factor (VIF) showed a value >10, the cell was not further analyzed because...
colinearity between eye and retinal slip might affect the results (see METHODS of Hirata and Highstein 2001). Head-related components were extracted by analyzing the neuronal and behavioral response during VOR (Eq. 2). Finally, the validity of the extraction method was confirmed by estimating the neuronal response during VORS and/or VORE paradigms and comparing the distribution and autocorrelation function of the residuals of those of the neuronal discharge during spontaneous eye movements (for further explanation, see Hirata and Highstein 2001). Only cells that passed these tests were used for further analysis. In the following text are the equations used for parameter extraction (see reference Blazquez et al. 2003).

\[
f_{\text{OKR}}(t) = \alpha \frac{dx(t + \tau_c)}{dt} + \beta_1 \frac{dx(t + \tau_c)}{dt} + \gamma x(t + \tau_c) + \alpha_1 \frac{dr(t + \tau_c)}{dt} + \beta_2 \frac{dr(t + \tau_c)}{dt} + \delta_{\text{OKR}} + \epsilon(t)
\]

where \( f \) is the firing rate, \( x \) is eye position, \( r \) is retinal slip, \( h \) is head position, \( \alpha \) is a DC gain, \( \beta_1 \) and \( \beta_2 \) are sensitivities to acceleration, velocity, and position, respectively. \( \alpha_1 \) is a variable gain, \( \gamma \) and \( \delta_{\text{OKR}} \) are sensitivities to eye, head, and retinal slip and head position, respectively, with \( \alpha \), \( \beta \), and \( \gamma \) denoting sensitivities to acceleration, velocity, and position, respectively. \( \epsilon \) is an error term or a residual component.

The preceding analysis methods provided information regarding the signal content in a single Y neuron at different VOR gain states. Because we have recently reported the signal content of Purkinje cells using identical experimental manipulation, we were able to directly compare both studies and extract additional information by assuming linear summation of input in Y neurons of the floccular and nonflocular pathways. An alternative method to extract eye- and head-velocity sensitivities from Y neuron firing is presented in supplementary material.

Model and estimation of signal transmission efficacies in other VOR pathways

In the previous section, we described how to calculate the overall sensitivities of Y neurons (eye, head, and retinal slip sensitivities) using the Y neuron discharge and the behavioral response. In this section, we describe how to estimate changes in the strength of specific information pathways by comparing the information obtained from Y neurons, Purkinje cells (Blazquez et al. 2003) and behavior within the context of the well-defined model for the vertical VOR circuitry (Hirata and Highstein 2001). In this model, the response obtained from Y neurons is used as a representative example of vertical FTNs. The model is evaluated in velocity mode as in other previous insightful models (Lisberger 1994; Miles and Lisberger 1981) and consists of six black boxes representing: vestibular pathway to flocculus (\( G_{\text{FL/VESTIB}} \)), direct vestibular inputs to FTNs (\( G_{\text{FTN/VESTIB}} \)), other vestibular pathway not going through flocculus nor FTNs (\( G_{\text{NON/FL/NON/FTN/VESTIB}} \)), pathway from flocculus Purkinje cells to FTNs (\( G_{\text{P/F/FTN}} \)), efference copy pathway that feeds motor signal back to flocculus (\( G_{\text{FL/COPY}} \)), and extraocular muscle plant (\( G_{\text{EOM}} \)). The assumptions of our model are similar to those used in previous general models of the VOR and consist of signals conveying different modalities are added linearly to produce Purkinje cell, Y neuron firing patterns, and eye movements; all the subsystems can be modeled as linear systems within a small stimulus range for relatively short period of time as currently employed; and velocity information alone is sufficient to account for system behavior in response to sinusoidal stimulations as velocity signal constitutes the dominant determinants of neuronal firing within the VOR system.

The values for most of the boxes shown in the model are known or can be estimated with the present data. Namely, our previous report on vertical Purkinje cells (Blazquez et al. 2003) provides \( G_{\text{FL/VESTIB}} \) and \( G_{\text{FL/COPY}} \) (Purkinje cell sensitivity to head and eye velocity, respectively). Other parameters in the model can be estimated by using results from vertical Purkinje cells and the current data obtained from Y neurons as follows. \( G_{\text{FTN/VESTIB}} \) is obtained as the ratio between Y neurons (FTN) eye-velocity sensitivity (\( \beta_v \) in Eqs. 1 and 3) and Purkinje cell eye-velocity sensitivity as Purkinje cells are the only source of eye signal to Y neurons (see RESULTS and DISCUSSION for further details). Calculating \( G_{\text{FTN/VESTIB}} \) is more complicated because Y neurons have two sources of head-velocity sensitivity (one arrives from the cerebellar flocculus and another from second vestibular neurons in the SVN) (Blazquez et al. 2000; Langer et al. 1985; Partsalis et al. 1995b). To calculate \( G_{\text{FTN/VESTIB/VESTIB}} \) we utilize a particular property found in the Purkinje cell population and described in Eq. 4B. Thus the ratio of eye-velocity versus head-velocity sensitivity of individual Purkinje cells is closely correlated with the VOR gain. This relationship can be expressed as Eq. 6 by using the schematic neuronal circuitry comprising Purkinje cells and FTNs (Y neurons) described in Fig. 4A.

\[
\phi/\lambda = \text{func}(G_{\text{VOR}}) = 0.94G_{\text{VOR}}^{-0.86}
\]

Where \( \phi \) and \( \lambda \) represent the eye- and head-velocity sensitivity of Purkinje cells and \( G_{\text{VOR}} \) represents the VOR gain at which Purkinje cells were recorded. Following the diagram of Fig. 4A, the response of FTN during VORd can be written as

\[
\text{FTN} = \omega H + \kappa P = \omega H + \kappa(\lambda H + \delta E) = (\omega + \kappa\lambda)H + \kappa\delta E
\]

Where FTN represents the Y neuron response, \( H \) the head velocity, \( P \) the Purkinje cell response, \( E \) the eye velocity. Transmission efficacies \( \omega \) and \( \kappa \) correspond to \( G_{\text{FTN/VESTIB}} \) and \( G_{\text{FTN/FM}} \) in Fig. 5, respectively. Furthermore, the coefficient \( \lambda \) for \( H \) can be expressed by using Eqs. 6 as \( [\omega + \kappa\lambda]/\text{func}(G_{\text{VOR}}) \), and because the model under consideration is in velocity mode, this coefficient corresponds to the head-velocity sensitivity of FTN (\( \beta_{\text{h}} \) in Eqs. 2 and 3), whereas \( \kappa\lambda \) corresponds to the eye-velocity sensitivity of FTN (\( \beta_{\text{v}} \) in Eqs. 1–3). Therefore we can estimate the value of \( \omega \) (\( G_{\text{FTN/VESTIB}} \) in Fig. 5) as \( \omega = \{\beta_h - \beta_v/\text{func}(G_{\text{VOR}}) \} \) (see APPENDIX for more details).

Additionally we assume \( G_{\text{PLAN}} \) to be 1 because we use the eye-velocity signal in place of the efference copy signal. \( G_{\text{NON/FL/NON/FTN/VESTIB}} \) is the unknown term and contains all the pathways that do not go through the flocculus or FTNs. The position vestibular pause (PVP) neurons located in the vestibular nuclei are included in this subsystem. We estimate the gain of this system \( G_{\text{NON/FLY/VESTIB}} \) to best-reproduce the experimental data (eye velocity, Purkinje cell modulation, and Y neuron modulation during VORd). A more detailed explanation of the model is presented in the APPENDIX.

RESULTS

Two squirrel monkeys (065 and 062) wore magnifying and/or minifying lenses for several months to either increase or decrease their VOR gains. The training order was different in each animal (065 first wore magnifying and then minifying lenses and vice versa for 062). VOR dark (d) gains were: normal, 0.85 ± 0.08 (minimum: 0.71, maximum: 0.97), high,
VOR, vestibulo-ocular reflex; VORd, vestibulo-ocular reflex in the dark; RSLP, retinal slip.

Low form this database (24 high gain; 20 normal gain; 24 low gain). As

FROM THIS DATABASE (24 HIGH GAIN; 20 NORMAL GAIN; 24 LOW GAIN). AS

Dynamic model of eye movement (average neuronal response versus eye movement during VORd

Two analysis methods were applied: a sinusoidal fit to the baseline firing rate (dc) between 80 and 110 spikes/s and previously reported by Partsalis et al. (1995a). Y neurons have a movement, and response to visual stimulation (OKS) as pre-

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movements, and response to visual stimulation (OKS) as pre-

analysis forms the basis for this report. Cells were identified by their location, characteristic discharge during spontaneous eye movements, and response to visual stimulation (OKS) as previously reported by Partsalis et al. (1995a). Y neurons have a

movements, and response to visual stimulation (OKS) as pre-

response to head and eye velocity in isolation. Neuronal recordings

of eye movement (Eqs. 1–3) (see Blazquez et al. 2003). However,

changes significantly after low-gain training (Fig. 2G, hollow arrow), and head-velocity sensitivity changes significantly after high-gain training (Fig. 2J, hollow arrow) (Blazquez et al. 2003).

Y neurons and floccular Purkinje cells are both gaze-velocity neurons with relatively equal magnitudes of head- and eye-velocity sensitivities in the naive animal. Thus both classes of neurons hardly modulate during VORD in the naive animal as the head and eye signals cancel each other. Purkinje cells monosynaptically inhibit Y neurons, presumably leading to mirror signal reciprocity in the naive condition. Therefore a lack of such reciprocity following training is highly significant as it emphasizes that the changes in Y neurons sensitivities are not merely the result of synaptic transfer of cerebellar changes in sensitivities. These results are further exemplified in the following text.

Changes in the eye- and head-velocity sensitivities of Y neurons after VOR adaptation

Eye- and head-velocity sensitivities were extracted using a multiple linear regression (cf. Eq. 3, METHODS). Of the 74 Y neurons recorded, 6 did not satisfy the criteria, hence, 68 neurons form this database (24 high gain; 20 normal gain; 24 low gain). As

in previous reports (Lisberger et al. 1994a,b; Partsalis et al. 1995a), only eye- and head-velocity sensitivities are considered because they constitute the dominant determinants of neuronal firing, and historically changes in these parameters have been used to analyze VOR motor learning. Y neuron eye-velocity sensitivity does not change after training (Fig. 2, B and G, filled arrows, Table 1), whereas head-velocity sensitivity increases significantly after low-gain (*P < 0.005) but not high-gain training (P > 0.91; Fig. 2, E and J, filled arrow, Table 1). In contrast, Purkinje cell eye-velocity sensitivity changes significantly after low-gain training (Fig. 2G, hollow arrow), and head-velocity sensitivity changes significantly after high-gain training (Fig. 2J, hollow arrow) (Blazquez et al. 2003).

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Figure 3A illustrates the gaze-velocity feature of naive Y neurons, namely equal eye- and head-velocity sensitivities (45° slope) with an intercept that passes through zero (fitting line). After high- and low-gain training, the slopes of the fitting lines change. These changes in slope are similar to those previously reported for Purkinje cells (Blazquez et al. 2003). However, unlike what happens for the Purkinje cells, for the Y neuron populations these fitting lines (Fig. 3A low, normal, and high gain) intercept the ordinate axes at different positions depending on the VOR gain. The latter, and the data presented in Fig. 2, suggest that changes in eye-/head-velocity sensitivity in Y neurons with training differs from that in Purkinje cells. This is illustrated in Fig. 3B. In the naive animal, the dotted (Purkinje cells) and solid (Y neurons) blocks are of equal magnitude but in opposite direction, again emphasizing a ratio of 1 between eye- and head-velocity sensitivity. After low-gain training, there is a much larger increase in the Purkinje cell eye/head ratio (1.8) than the decrease in its Y neuron counterpart (0.5) as exemplified by the relative sizes of the solid and dotted boxes in Fig. 3B. A smaller, but similarly disproportionate change in

TABLE 1. Y neuron sensitivities and modulation during VORd in the naive and adapted animal

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<tbody>
<tr>
<td>High gain</td>
<td>1.43 ± 0.14</td>
<td>0.44 ± 0.31</td>
<td>184.55</td>
<td>0.933 ± 0.28</td>
<td>−0.33 ± 0.7229</td>
<td>0.023 ± 0.0254</td>
<td>0.89 ± 0.4</td>
<td>0.055 ± 0.4</td>
<td>0.32 ± 0.18</td>
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<tr>
<td>Normal gain</td>
<td>0.85 ± 0.08</td>
<td>0.29 ± 0.31</td>
<td>23.81</td>
<td>0.786 ± 0.34</td>
<td>−0.65 ± 0.88</td>
<td>0.010 ± 0.0150</td>
<td>0.9 ± 0.4</td>
<td>0.068 ± 0.04</td>
<td>0.438 ± 0.23</td>
</tr>
<tr>
<td>Low gain</td>
<td>0.51 ± 0.08</td>
<td>0.96 ± 0.28</td>
<td>17.55</td>
<td>0.730 ± 0.46</td>
<td>−0.71 ± 0.86</td>
<td>0.018 ± 0.0292</td>
<td>1.24 ± 0.35**</td>
<td>0.11 ± 0.05**</td>
<td>0.469 ± 0.31</td>
</tr>
</tbody>
</table>

Response of Y neurons during VORD and sensitivities to eye, head, and retinal slip parameters in the normal-gain, low-gain, and high-gain-adapted animal. Values are Means ± SD. Statistical comparison was shown using an ANOVA single factor (*P < 0.05 and **P < 0.01 compared to the normal gain population).
eye-/head-velocity sensitivity can also be seen for the high-gain case. These results are also plotted as the eye/head contributions to the overall firing of Y neuron (solid boxes) and Purkinje cell (dotted boxes) versus VOR gain in Fig. 3, D and E. The monotonic increase in slope of the ratio (eye-velocity sensitivity/head-velocity sensitivity) in Y neurons parallels increasing VOR gain and is most likely causal in increasing VOR gain as the Y neuron output is a direct contributor to the motor output.

Thus even though Purkinje cells are the major input to the dorsal Y group, synaptic transfer of any learned changes in the flocculus alone cannot explain changes in Y neuron discharge following learning.

Changes in other Y neuron parameters

Analysis of Y neuron discharge also suggests changes in the head and retinal slip acceleration sensitivity after low-gain training (cf. Fig. 2). No significant changes were observed in other parameters, including eye position (cf. Table 1). A tendency for a monotonic increase in baseline firing in light and dark with gain increase from low to high was also noted in this study and agrees with previous reports (Partsalis et al. 1995a).

Changes in the nonfloccular head-velocity pathway to Y neurons following learning

The nonfloccular head-velocity pathway to Y neurons consists of two synapses, one from the VIIIth nerve to secondary vestibular neurons in the superior vestibular nucleus, also called Y projecting neurons (YPNs), and a second from YPNs to Y neurons. For simplicity, YPNs are not represented in our circuit diagram (Fig. 4A); however, we will call this disynaptic pathway to Y neurons the YPN pathway (Fig. 4A, or $G_{FTN\_vestib}$ in Fig. 5).
As illustrated in Fig. 4A, the immediately prenuclear sources of eye and head velocity to Y neurons are flocculus Purkinje cells that fire relative to both head and eye velocity (κ) and YPNs that encode head velocity alone (ω) (Blazquez et al. 2000). Therefore the head-velocity signal to Y neurons has two origins, whereas eye-velocity information arrives exclusively from floccular input (Partsalis et al. 1995b; Rambold et al. 2002). Experimental evidence and modeling (Lisberger 1994; Partsalis et al. 1995b; Rambold et al., 2002) support this view (Fig. 4A). Thus the head-velocity sensitivity of individual Y neurons (βh) results as the sum of their floccular and nonfloccular head-velocity inputs (ω and κλ) and the eye-velocity sensitivity of individual Y neurons (βe) is determined by their floccular input (κφω). In addition, Fig. 4B indicates that Purkinje cells show an exponential relationship between their eye velocity/head velocity (ω/λ) and VOR gain. All this information can be used to further extract the value of κλ and ω (see METHODS and APPENDIX for further explanation). Figure 4, C and D, illustrates the changes in the nonfloccular head-velocity inputs (ω) to Y neurons for each cell versus the VOR gain. Note that ω increases after low gain and decreases after high-gain adaptation (normal gain: +0.18, low gain: +0.76, and high gain: −0.23 spike/s per °/s).

Recall Fig. 3B, which suggests that in low gain, Y neuron firing is dominated by head velocity and, conversely, by eye velocity in high gain. In contrast, Purkinje cell firing is dominated by eye velocity in low gain and by head velocity in high gain. However, the slopes (Fig. 3A) for Y neurons are very similar to those for Purkinje cells (Blazquez et al. 2003). We suggest that these results can be explained by a change in the strength of the nonfloccular vestibular pathways (ω) to Y neurons. This change adds a dc term to the head-velocity sensitivity of Y neurons for the low-gain animal (the intercept is positive), whereas it works in the opposite direction for the high-gain animal (the intercept is negative).

**Changes in the Purkinje cell inputs to Y neurons (κ)**

Two methods are used to obtain the value of κ (cf. APPENDIX for details). First, the most obvious way to extract κ is by comparison of the measured eye-velocity sensitivities of Purkinje cells (φω) and Y neurons (βω) before and after motor learning

\[ \beta_ω = \phiω + κλ \]

This comparison indicates a decrease in the efficacy of κ after low-gain adaptation and little change after high-gain adaptation (see supplementary Fig. 4).

A second method uses the value of ω calculated in the preceding text (see APPENDIX)

\[ β_ω = ω + κλ \]

The value of κ (the nonfloccular YPN signal) can thus be obtained in the normal and adapted animal. One caveat is that...
the eye- and head-velocity sensitivity of Y neurons changes with VOR gain and the populations of Y neurons and Purkinje cells were recorded at slightly different gains. Hence, the exact values of $\kappa$ might be slightly different from those obtained here; however, the directional changes should be identical.

Postulated changes in the head-velocity pathway to PVP cells

There is another pathway subserving the VOR that neither goes through flocculus nor receives flocculus input. This third pathway is thought to be composed of vertical PVP neurons (McCrea and Highstein 1987) and is illustrated as $G_{\text{non-FL/non-FTN_vestib}}$ in Fig. 5. The basic architecture of the model is similar to that proposed by Lisberger (1994) and Tabata et al. (2002) but distinct in that the third pathway is explicitly presented (Hirata and Highstein 2001). In the model, each box represents the transfer function of each pathway, and $\lambda$, $\phi$, $\kappa$, and $\omega$ in Fig. 4A correspond to $G_{\text{FTN_vestib}}$, $G_{\text{FTN_vestib}}$, $G_{\text{FTN_vestib}}$, and $G_{\text{FTN_vestib}}$, respectively (see APPENDIX for more detailed description of the model). Although we did not record PVP activity, the gain of the corresponding transfer function ($G_{\text{non-FL/non-FTN_vestib}}$) can be estimated from the experimental data from Y neurons and Purkinje cells at each VOR gain state under the anatomically plausible constraints of the model (see APPENDIX).

Evaluation of the role of cerebellar learning in memory retention (model simulation)

Because the expression of new motor memories results not only from changes in signal transmission efficacies within the network but from the network architecture itself, experimental results that use lesions that change the network configuration can be misleading. This is even more important when lesions eliminate a feedback loop as occurs with flocculectomy.

Our model simulation can reproduce the experimental results obtained in the normal and the chronically adapted states (Table 2 and Table 4). On the basis of the validity of this model, we designed an alternative strategy to evaluate the role of the cerebellum in motor learning. This strategy consists of simulating the VOR behavior by assuming no cerebellar plasticity (eye- and head-velocity sensitivities of Purkinje cells equal to those obtained in the normal gain animal) at the same time that the sensitivities of brain stem neurons are set to those values observed experimentally after high- and low-gain adaptation. We believe that this is a more correct strategy to account for the effect of cerebellar plasticity in memory retention because it leaves the feedback loop intact. This simulation predicts a VOR gain of 4.1 after high-gain adaptation and 0.3 after low-gain adaptation (Tables 3 and 4). Because the experimentally observed VOR gains were ~1.59 and 0.47 for high- and low-gain training, respectively, this result suggests that cerebellar plasticity prevents the system gain from overshooting and works together with brain stem plasticity to tune the gain to the appropriate values.

Interpretation of flocculectomy (model simulation)

Flocculectomy in the adapted animal has two immediate effects: it will prevent the expression of cerebellar learning (changes in $G_{\text{FL_ecopy}}$ and $G_{\text{FTN_vestib}}$ in Fig. 5), and it will eliminate the enhancement effects of the feed-back loop (formed by $G_{\text{FL_ecopy}}$ and $G_{\text{FTN_vestib}}$ in Fig. 5). Our model suggests that flocculectomy will result in no changes in VOR gain in the normal animal because eye- and head-related signals are properly balanced at the Purkinje cell level canceling each other and resulting in no net modulation. However, this is not the case for the high- and low-gain states. Our simulations predict that flocculectomy performed after chronic VOR motor learning will result in a partial loss of new VOR gain (Table 3). However, the mechanism that maintains the
new VOR gain after the flocculectomy is no longer identical to that in the intact animal because the system only works in the feed-forward mode. Our simulation results agree with those obtained experimentally (Broussard and Kassardjian 2004; Luebke and Robinson 1994; Partsalis et al. 1995). Thus it appears that in the intact animal, changes in Purkinje cell sensitivities are necessary to compensate for the effects of the feedback loop.

FIG. 5. Model system used to represent the VOR circuitry. Head-velocity information travels via 3 separate pathways, 1 pathway (G_{FL_vestib}) conveys head-velocity information to Purkinje cell where is added with information of the ongoing movement (G_{FL_ecopy}) and sent to its target neurons in the brain stem (G_{P_FTN}). These neurons in the brain stem receive a 2nd pathway (G_{non-FL_vestib}) that conveys head-velocity information from the VIIIth nerve or from interneurons in the vestibular nucleus. A 3rd head-velocity pathway (G_{non-FL/non-FTN_vestib}) bypasses the cerebellum and FTNs. Signals from G_{non-FL/non-FTN_vestib} and FTNs combine to generate the appropriate motor command to move the eyes (G_{plan}), which in turn is sent back to the cerebellum. Purkinje cells and FTNs are shown as nodes (○) in the circuitry. Vestibular pathway that does not go through FTNs is represented here as G_{non-FL/non-FTN_vestib} H, head velocity; X, motor output as eye velocity (see APPENDIX for further explanation). The feed back loop comprises G_{FL_ecopy} and G_{P_FTN} and the feed forward loop comprises 3 parallel pathways; 1, G_{FTN_vestib} and G_{plant}; 2, G_{FL_vestib} G_{P_FTN} and G_{plant}; 3, G_{non-FL/non-FTN_vestib} and G_{plant}.

DISCUSSION

Summary

Our results suggest that the storage of new VOR memories results from plastic changes in multiple sites located in the cerebellar cortex and the brain stem. In contrast, previous models assumed the existence of two main plastic sites for VOR motor learning, one located in the cerebellar cortex and another located in the brain stem FTNs (Lisberger 1994). Previously it was suggested that VOR learning equated to changes in the head-velocity pathway and in the efference copy pathway to the flocculus (Blazquez et al. 2003). In this report, we suggest that the signal transfer between cerebellar cortex and Y neurons (the putative vertical equivalent of horizontal FTNs) is also under regulatory control. We also suggest that the signal transmission efficacy from vestibular sensory signal to Y neurons is plastic. Additionally our simulation suggests that motor learning in the vertical VOR requires plastic changes in noncerebellar, non-Y neuron pathways (presumably at the PVPs). Our model simulations also caution that the conclusions drawn following floccular inactivation can be misleading.

Reliability of the analysis methods

The multiple linear regression analysis currently employed for Y neurons is the same as in the previous analyses for

<table>
<thead>
<tr>
<th>TABLE 2. Model parameters and estimation of G_{non-FL/non-FTN_vestib}</th>
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<tbody>
<tr>
<td>VOR Gain</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>Low (0.42)</td>
</tr>
<tr>
<td>Norm (0.83)</td>
</tr>
<tr>
<td>High (1.61)</td>
</tr>
</tbody>
</table>
Purkinje cell firing patterns during the same experimental paradigms Blazquez et al. 2003; Hirata and Hightstein 2001. A criticism for this analysis method is the colinearity among the regressors (Slinker and Glantz 1985), especially between eye velocity and retinal slip velocity. If these two signals are colinear, estimated parameters (sensitivities) are not reliable. However, colinearity is not the case even when a sinusoidal stimulus is employed because eye velocity deviates from a sinusoid, and thus retinal slip calculated as stimulus sinusoid minus eye velocity is not sinusoidal nor similar to eye velocity. This is especially assured when we do not average these signals over stimulus cycles. We also evaluate potential colinearity by the VIF and a cross-validation check (see METHODS) (Hirata and Hightstein 2001). Another caveat may be in estimating neuronal head parameters following VOR training by the extraction of the eye signal in the light with the head fixed and the subsequent subtraction of this number from the total neuronal modulation during the VORd. Critics have suggested that this strategy is inappropriate as the animal might be in different states in the light and dark. We argue that whatever the inherent state differences might be, they should be consistent across VOR gains. In support, floccular inactivation removes any eye-velocity sensitivity from FTNs at any gain (Langer et al., 1985; Partsalis et al. 1995b; Rambold et al. 2002; Takemura et al. 2001), and head-velocity sensitivity obtained by VOR cancellation in the light generally agrees numerically with that obtained with the subtraction method (Lisberger et al. 1994a,b).

Head-velocity sensitivity of nonflocculus origin and signal transmission efficacy between Purkinje cell and Y neurons (ω and κ in Fig. 4A) were both estimated by using Y neuron and Purkinje cell data that were recorded during the same paradigms in the same experimental setup, but from different monkeys at different VOR gains. Large SDs of VOR gains and limited samples of Purkinje cell data potentially cause an unreliable estimation of these values. We can avoid this problem for ω by employing the new finding that the ratio of eye- and head-velocity sensitivities of Purkinje cells tightly follows an exponential function of the VOR gain at which the cell was recorded (Fig. 4B, see APPENDIX). For the estimation of κ, we employed Eq. A2 in which eye-velocity sensitivity, not head-velocity sensitivity, of Purkinje cells was used. Although the absolute magnitude of the adapted high gain in Purkinje cells was slightly different from that in Y neurons, eye-velocity sensitivity in Purkinje cells does not change from normal to high gain. Thus Eq. A2 should provide a good estimation for the κ of each Y neuron even for the high-gain state.

Another estimation currently performed is for the gain of nonflocculus, non-FTN vestibular pathway (the 3rd, PVP pathway). We estimated the gain of this pathway at different VOR gains so that we could reproduce the averaged Y neuron modulation, Purkinje cell modulation, and eye velocity during VORd at each gain state. The estimation was performed under the restriction of the model structure illustrated in Fig. 5, which is anatomically based. The reproduced neural modulations (Y neurons and Purkinje cells) and eye movement are very close to those experimentally observed at each gain state (Table 4), assuring the reliability of the gain estimations.

Finally, the model used to explain the new behavior (Fig. 5) is based on an interpretation of the system in velocity mode only. Further studies involving recording from the neuronal integrator (for example, nucleus prepositus hypoglossi and interstitial nucleus of Cajal) should be carried out to fully understand how canal-related signal (velocity) are converted into eye movements (dominated by eye position) in the normal and adapted animal.

Differential changes in eye-velocity sensitivity of Purkinje cells and Y neurons

Neuronal recordings and lesion experiments indicate that the eye-velocity component of Y neuron discharge is provided exclusively by their floccular input. Rambold et al. (2002) showed that pursuit behavior is abolished after removal of the flocculus and more importantly Partsalis et al. (1995b) showed that removal of the ipsilateral flocculus eliminates the eye-velocity component of the firing rate of ipsilateral Y neurons (e.g., they stop modulating during OKR). This premise has been used by us and others to simulate the functioning of the VOR circuitry (Blazquez et al. 2003; Hirata and Hightstein 2001; Ito 2002; Lisberger 1994). The population of vertical Purkinje cells in the flocculus appears to be more homogeneous than its horizontal counterpart. Thus all vertical Purkinje cells described have a down-eye- and a down-head-velocity signal (Blazquez et al. 2003; Hirata and Hightstein 2001). In contrast, horizontal Purkinje cells could have only eye-related information or a combination of eye- and head-related information (Belton and McCrea 2000). In addition, a single flocculus contains horizontal Purkinje cells with left- and rightward-related signals. Thus for the vertical system, changes in the eye-velocity sensitivity of Purkinje cells (Blazquez et al. 2003) could potentially be transferred to Y neurons. However, because these changes are notably different from those observed in Y neurons, this transfer is questionable. Purkinje cells increase their eye-velocity sensitivity on average by 0.93 spikes/s per °/s after low-gain training and by 0.09 spikes/s per °/s after high-gain training (Blazquez et al. 2003). In contrast, Y neurons decrease their eye velocity sensitivity by 0.07 after low-gain training and increase it by 0.19 after high-gain training. These results can explain how the large changes in eye-velocity sensitivity observed experimentally in the cerebellar

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**Table 3. VOR gain after model modifications**

<table>
<thead>
<tr>
<th>Gain State</th>
<th>No Plasticity in Flocculus</th>
<th>No Plasticity Between PC &amp; YN</th>
<th>Flocculectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (0.42)</td>
<td>0.30</td>
<td>0.65</td>
<td>0.53</td>
</tr>
<tr>
<td>Normal (0.83)</td>
<td>–</td>
<td>–</td>
<td>0.90</td>
</tr>
<tr>
<td>High (1.61)</td>
<td>4.11</td>
<td>1.41</td>
<td>1.32</td>
</tr>
</tbody>
</table>

PC, Purkinje cells; YN, dorsal Y group neurons.

**Table 4. Model performance**

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC modulation, spikes/s per °/s</td>
<td>$-0.20 \pm 0.15$</td>
<td>$-0.37 \pm 0.23$</td>
<td>$0.42 \pm 0.21$</td>
</tr>
<tr>
<td>YN modulation, spikes/s per °/s</td>
<td>$0.29 \pm 0.31$</td>
<td>$0.92 \pm 0.34$</td>
<td>$-0.56 \pm 0.27$</td>
</tr>
<tr>
<td>VOR gain</td>
<td>$0.90 \pm 0.10$</td>
<td>$0.47 \pm 0.10$</td>
<td>$1.59 \pm 0.26$</td>
</tr>
</tbody>
</table>

Bold face, experiment (Mean ± SD); italic, model.
cortex (Purkinje cells) after low-gain adaptation do not affect the smooth pursuit behavior (Blazquez et al. 2004). Furthermore, nonmonotonic changes in eye- and head-velocity sensitivities at the Purkinje cell level are converted to a monotonic change at the level of Purkinje cell-Y neuron synapses (Lisberger 1994).

We hypothesize a modification in the strength of synaptic transfer between Purkinje cells and Y neurons. We estimate that the strength of the synapses decreases 53% after low-gain training but increases only ~9% after high-gain training (cf. APPENDIX). Similar inhibitory synapses exist on Purkinje cells target neurons in the deep cerebellar nuclei, where LTD and LTP mechanisms have been suggested (Hansel et al. 2001). Additionally, the existence of nonsynaptic forms of plasticity has been demonstrated (for a recent review, see Zhang and Linden 2003). Nelson and colleagues have described changes in the spontaneous discharge of vestibular neurons and suggested that they might be evoked by inhibitory synaptic stimulation suggesting a nonsynaptic form of plasticity (Nelson et al. 2003).

An alternative theory is that synapses might be subjected to nonlinear effects such as saturation. Gauck and Jaeger (2003) have shown that the information transfer from Purkinje cells to target neurons in the deep cerebellar nuclei is complex and depends highly on the type of spiking activity of Purkinje cells. However, we find it unlikely that the differential changes observed between Purkinje cells and Y neurons are the consequence of a saturation effect because Y neurons are capable of larger modulations during sinusoidal OKS than those used in this study (see supplementary Fig. 5). We favor the view that plastic changes (of synaptic origin) are the main candidate to account for the differential changes in Purkinje cells and Y neurons after VOR adaptation. These results are consistent with the hypothesis that the flocculus provides a teaching or instructional signal that is causal to changes in synaptic weight of the inputs to its target neurons. This is another example of a type of heterosynaptic or Hebbian learning.

Changes in the nonfloccular pathway (YPN pathway) to Y neurons

Our data show that there is a directional change in the nonfloccular head velocity sensitivity of Y neurons in the low- and high-gain animal. To extract the nonfloccular head-velocity sensitivity we use a novel observation that the head- and eye-velocity sensitivities of Purkinje cells change together maintaining a ratio exponentially related to the VOR gain (see APPENDIX). Results are in agreement with the literature that in the signal transmission from Purkinje cells to FTNs reported here for the vertical system might also happen for the horizontal system to maintain normal pursuit behavior (Blazquez et al. 2004). And we also hypothesize that the asymmetric reversibility effect reported by Boyden and Raymond (2003) occurs in both the horizontal and vertical system.

Floccular role in VOR motor learning and interpretation of flocculectomy

The changes observed in Purkinje cell sensitivities and in the efficacy of the signal transmission between Purkinje cells and FTNs are critical to maintain the new VOR gain and seem appropriate to be causal for the new behavior. Simulations predict that VOR gain will be severely compromised without cerebellar plasticity (gain will over-adapt for both high- and low-gain training). We offer a simple explanation, suggesting that, at least for the vertical VOR, the plastic changes observed not only at the Purkinje cell level but also at the cerebellar synaptic output work to compensate the effects of the feedback loop during VORd behavior. Therefore these changes are necessary to maintain the new behavior. Flocculectomy is, however, a different situation wherein removal of the flocculus will have two consequences: it will eliminate the influence of the plastic changes that have taken place in the cerebellum and it will remove the effect of the feed-back loop that works by amplifying any mismatch between the head signal and the efference copy signal at the Purkinje cell level. Thus the behavior that remains after flocculus removal results from a different mechanism than that in the normal animal. Namely, in...
the intact animal, changes in the feed-forward pathways after VOR adaptation are enhanced and compensated by the changes in feed-back system, whereas in the flocculectomized animal, only the consequences of changes in the feed-forward system are manifested.

Changes in other parameters

In the present report, we interpret the vertical VOR circuitry in velocity mode as was done for the horizontal system; however, further studies are needed to help us comprehend VOR motor learning using all the signals available (not only velocity and not only head and eye signals). Neuronal recording and modeling simulations suggest that changes must occur also in the integrator pathway (Blazquez et al. 2003). Additionally, the present data on Y neurons and previous reports on Purkinje cells indicate changes in other parameters in addition to eye- and head-velocity sensitivities. We do not have yet a valid explanation for these changes. To properly interpret these changes, we need to study the neuronal response during different paradigms than those used in this study (sinusoidal stimulation at a single frequency). Changes in these parameters could also indicate changes in nontraditional plastic brain sites, like have been shown for saccade adaptation (Takeichi et al. 2005).

APPENDIX

Method to quantify the nonfloccular pathway (YPN pathway) to Y neurons (ω)

The head-velocity signal present in Y neurons results from two input pathways as illustrated in Figs. 4A and 5, namely the floccular input and the nonfloccular, direct VIII nerve input via SVN interneurons (called here YPN pathway to Y neurons) (Blazquez et al. 2000). From the figures, the head-velocity sensitivity of Y neurons can be described as follows

$$\beta_h = \omega + \kappa \lambda$$  \hspace{1cm} (A1)

To separately evaluate the head signal in Y neurons arriving from each pathway, we need to know the signal transmission efficacy from flocculus Purkinje cells to Y neuron (κ) and Purkinje cell head sensitivity (λ). κ can be estimated by using eye-velocity sensitivities of Purkinje cells (β) and Y neurons (βh) because the source of eye-velocity signal in Y neuron is exclusively from flocculus Purkinje cells (Partsalis et al. 1995b) at least at 0.5 Hz of OKS we currently and previously (Blazquez et al. 2003) used. Namely, Y neurons eye-velocity sensitivity (βh) is equal to Purkinje cell eye-velocity sensitivity (β) multiplied by κ, thus κ is given as

$$\kappa = \beta_h / \beta$$  \hspace{1cm} (A2)

However, to apply this formula, Y neuron and Purkinje cell eye-velocity sensitivities should be obtained at the same VOR gains because both sensitivities depend on VOR gain. We could curve-fit the Purkinje cell eye-velocity sensitivities versus VOR gain characteristics and obtain Purkinje cell eye-velocity sensitivity at the VOR gain where the Y neuron under consideration was recorded. However, the Purkinje cell eye-velocity sensitivity is highly variable for cell by cell (Blazquez et al. 2003), thus the estimated eye-velocity sensitivity for the group will have a long SD. In contrast, we currently demonstrated that the ratio of Purkinje cell eye-velocity sensitivity and head-velocity sensitivity, for individual cells, is tightly related to animal’s VOR gain as follows (also as illustrated in Fig. 4B)

$$\phi / \lambda = \text{func}(G_{\text{VOR}}) = 0.94(G_{\text{VOR}})^{-0.96}$$  \hspace{1cm} (A3)

Thus the head-velocity signal of Purkinje cells (λ) could be expressed as

$$\lambda = \phi / \text{func}(G_{\text{VOR}})$$  \hspace{1cm} (A4)

Because the only source of eye-velocity sensitivity of Y neurons arrives via their floccular Purkinje cell inputs, the relationship shown in Eq. A4 will be maintained at the level of the Y neurons as expressed in the following text (see Fig. 4A)

$$k\lambda = k\phi / \text{func}(G_{\text{VOR}})$$  \hspace{1cm} (A5)

The term kφ corresponds to the eye-velocity sensitivity observed at the Y neuron level (βh) and Eq. A5 could be represented as

$$k\lambda = \beta_h / \text{func}(G_{\text{VOR}})$$  \hspace{1cm} (A6)

Using Eqs. A6 and A1, we can reliably separated the head-velocity sensitivity of Y neurons without using κ nor λ as follows

$$\beta_h = \omega + \beta / \text{func}(G_{\text{VOR}})$$  \hspace{1cm} (A7)

Therefore Y neuron sensitivity to nonfloccular head velocity (ω) is given as

$$\omega = \beta_h - \beta / \text{func}(G_{\text{VOR}})$$  \hspace{1cm} (A8)

Note that estimation of kλ and w is as reliable as the goodness of fit of Eq. A3 (Fig. 4B).

Method to estimate the transmission efficacy from Purkinje cells to Y neurons (κ).

With the data at hand we can use several methods to extract the value of κ. These methods assume that Y neuron sensitivities to head and eye velocity can be understood as a linear summation of its floccular and nonfloccular inputs. We use Fig. 4A to define the architecture of the network. Results from both methods presented here show similar changes in the value of κ after VOR motor learning.

METHOD 1: COMPARISON OF THE AVERAGE EYE-VELOCITY SENSITIVITY OF Y NEURONS AND PURKINJE CELLS BEFORE AND AFTER VOR MOTOR LEARNING. We use Eq. A2 before and after VOR motor learning to obtain the value of κ. Hence we only need the sensitivities to eye velocity of Y neuron (Table 1) and Purkinje cell (Table 2 in Blazquez et al. 2003) that were extracted directly from the neuronal response during OKR (see METHODS).

Low gain animal

$$\beta_h = \phi \kappa$$

$$0.73 = -2.0781\kappa$$

$$\kappa = -0.3513$$

Normal gain animal

$$0.786 = -1.0785\kappa$$

$$\kappa = -0.7288$$

High gain animal

$$0.933 = -1.1759\kappa$$

$$\kappa = -0.7934$$

κ low/κ normal: 48.20% κ high/κ normal: 108.86%

METHOD 2: COMPARISON OF THE AVERAGE EYE- AND HEAD-VELOCITY SENSITIVITY OF Y NEURONS AND PURKINJE CELLS BEFORE AND AFTER VOR MOTOR LEARNING. We use Eq. A1 before and after VOR motor learning to obtain the value of κ. Hence, we have to know the head-velocity sensitivities of Y neurons and Purkinje cell (extracted during VORd), and the head-velocity sensitivity of the YPN.
pathway to Y neurons (these values were calculated in the text preceding Eq. A8).

Low gain animal

\[ \beta_h = \omega + \kappa \lambda \]
\[ 1.24 = -1.1783\kappa + 0.76 \]
\[ \kappa = -0.4074 \]

Normal gain animal

\[ \beta_h = \omega + \kappa \lambda \]
\[ 0.9 = -0.9845\kappa + 0.18 \]
\[ \kappa = -0.7313 \]

Normal gain animal

\[ \beta_h = \omega + \kappa \lambda \]
\[ 0.89 = -1.53\kappa - 0.23 \]
\[ \kappa = -0.7315 \]
\[ \kappa \text{ low/}\kappa \text{ normal: 56\%} \]
\[ \kappa \text{ high/}\kappa \text{ normal: 100\%} \]

**Model formulation and simulation**

**SYSTEM EQUATIONS.** The system equations of the model illustrated in Fig. 5 are described as follows in the Laplace domain

\[ P(s) = H(s)G_{\text{FL_vestib}}(s) + (\text{FTN}(s) + H(s)G_{\text{modY/vestibY}}(s))G_{\text{FL_ecopy}}(s) \]
\[ \text{FTN}(s) = P(s)G_{\text{P-FTN}}(s) + H(s)G_{\text{FTN_vestib}}(s) \]
\[ X(s) = (\text{FTN}(s) + H(s)G_{\text{modY/vestibY}}(s))G_{\text{FTN_vestib}}(s) \]  

(A9)

where \( s \) denotes the Laplace operator, and \( P, \text{FTN}, X, \) and \( H \) denote Laplace transformation of Purkinje cell firing pattern \( p(t) \), FTN firing pattern \( f(t) \), eye velocity \( x(t) \) and head velocity \( h(t) \), respectively. As the stimuli used in our current experiments are sinusoids at a single frequency (0.5 Hz), gains (scalar values) represent the preceding transfer functions. Note that the model description in Eq. A9 does not assume any signal class for \( h(t), x(t), f(t), \) and \( p(t) \), i.e., nonsinusoidal signals are applicable. The only assumption made is that each subsystem described by a transfer function is time invariant. To simplify the manipulation of equations in the following text, we assume the amplitude of head velocity is 1. \( G_{\text{FL_ecopy}} \) is set to be constant 1. This is due to the fact that we use eye movement for effference copy signal, and thus its gain is absorbed by \( G_{\text{FL_vestib}} \). The preceding equations then can be expressed in mutually independent form as follows

\[ \hat{p} = G_{\text{FL_vestib}} \]
\[ + \frac{G_{\text{FL_vestib}}G_{\text{FL_ecopy}}G_{\text{P-FTN}} + G_{\text{FL_vestib}}G_{\text{FL_vestib}}G_{\text{modY/vestibY}}}{1 - G_{\text{FL_ecopy}}G_{\text{P-FTN}}} \]
\[ \hat{f} = G_{\text{FL_vestib}}G_{\text{P-FTN}} + G_{\text{FL_vestib}} \]
\[ + \frac{G_{\text{FL_vestib}}G_{\text{P-FTN}}(G_{\text{FL_vestib}}G_{\text{P-FTN}} + G_{\text{modY/vestibY}} + G_{\text{modY/vestibY}})}{1 - G_{\text{FL_ecopy}}G_{\text{P-FTN}}} \]
\[ \hat{x} = \frac{G_{\text{FL_vestib}}G_{\text{P-FTN}} + G_{\text{modY/vestibY}}}{1 - G_{\text{FL_ecopy}}G_{\text{P-FTN}}} \]  

(A10)

As this is a model in velocity mode, we can substitute the gain values with the velocity sensitivities or transmission efficacies of corresponding neuronal sites: \( G_{\text{FL_vestib}} \) and \( G_{\text{FL_ecopy}} \) are Flocculus Purkinje cell head- and eye-velocity sensitivities, respectively, whereas \( G_{\text{FTN_vestib}} \) and \( G_{\text{P-FTN}} \) are, respectively, Y neuron sensitivity to head velocity of nonflocculus origin and transmission efficacy from Purkinje cells to Y neuron that are estimated as mentioned in the preceding text.\(^2\)

\(^2\) As the mean VOR gains are slightly different for monkeys recorded Purkinje cells and Y neurons, we used the VOR gains for Purkinje cells (Purkinje cell VOR gains: 0.90, 0.47, 1.59 for normal-, low-, and high-gain animals, respectively. Y neuron VOR gains: 0.85, 0.51, 1.43). The reason for using Purkinje cell values is that parameters related to Y neurons are calculated based on the parameters of Purkinje cells. Thus we interpolated Y neuron parameters along the VOR gain axis and estimated their values at Purkinje cell VOR gains (see Table 2 for parameter values used in the model).

**Determination of the optimum \( G_{\text{non-FL/non-FTN_vestib}} \)**

Although we are not able to determine the value of \( G_{\text{non-FL/non-FTN_vestib}} \) based on experimental data currently available, we are able to estimate it so that each node of the model (Purkinje cell, FTN firing modulation, and eye velocity) best reproduces corresponding experimental data. The optimum value for \( G_{\text{non-FL/non-FTN_vestib}} \) can be determined by minimizing the following object function

\[ \eta = (p - \beta)^2 + (f(tn - ftn)) + (x - \delta)^2 \]

(A11)

where \( p, ftn \), and \( x \) are experimental Purkinje cell, Y neuron firing modulation and eye velocity, respectively, while those with circumflex represent corresponding model output. We used the results of sinusoidal fit to the Purkinje cell and Y neuron firing pattern averaged over stimulus cycles calculated in the current (see METHODS) and previous studies for the values of \( p \) and \( ftn \), respectively (see footnote 2). For the value of \( x \), the mean VOR gain of monkeys at each VOR gain state are used. The object function Eq. A11 can be minimized by solving the following equation

\[ \frac{\partial \eta}{\partial G_{\text{modY/vestibY}}} = 0 \]  

(A12)

The optimal value for \( G_{\text{non-FL/non-FTN_vestib}} \) that satisfies Eq. A12 is uniquely determined as follows

\[ G_{\text{non-FL/non-FTN_vestib}} = \frac{\hat{p}B + yD + xF + AB + CD + EF}{B^2 + D^2 + F^2} \]  

(A13)

where

\[ A = -G_{\text{FL_vestib}} - \frac{G_{\text{modY/vestibY}}G_{\text{P-FTN}} + G_{\text{FTN_vestib}}}{1 - G_{\text{FL_ecopy}}G_{\text{P-FTN}}} G_{\text{FL_ecopy}} \]
\[ B = -\frac{G_{\text{FL_ecopy}}}{1 - G_{\text{FL_ecopy}}G_{\text{P-FTN}}} \]
\[ C = -(G_{\text{FL_vestib}}G_{\text{P-FTN}} + G_{\text{FTN_vestib}})(1 + \frac{1}{1 - G_{\text{FL_ecopy}}G_{\text{P-FTN}}} G_{\text{FL_ecopy}}G_{\text{P-FTN}}) \]
\[ D = -\frac{G_{\text{FL_ecopy}}G_{\text{P-FTN}}}{1 - G_{\text{FL_ecopy}}G_{\text{P-FTN}}} \]
\[ E = -G_{\text{FL_vestib}}G_{\text{P-FTN}} + G_{\text{FTN_vestib}} \]
\[ F = -\frac{1}{1 - G_{\text{FL_ecopy}}G_{\text{P-FTN}}} \]

The values of the optimized \( G_{\text{non-FL/non-FTN_vestib}} \) for normal-, low-, and high-gain states are shown in Table 2 together with those of other subsystems. In the first column, the simulated VOR gain values are also indicated in parenthesis.

**Model simulation**

**INTACT BEHAVIOR.** We implemented the model on the MATLAB (Mathworks) SIMULINK (Supplementary material). To run the model, MATLAB and SIMULINK must be installed. The results of model simulation for normal-, low-, high-gain animals are summarized in Table 2. The model reproduces quantitatively the experimental Purkinje cell, Y neuron firing modulations and eye velocity at each VOR gain state. Note that all the model outputs are within one SD of the corresponding experimental data.\(^3\)

\(^3\) To run this simulation, open model_vor1.mdl from SIMULINK and select run from the simulation menu. Green scopes connected to each node of the
NO PLASTIC CHANGES IN FLOCCULUS. To evaluate the role of flocculus in memory retention after VOR adaptation, we set the values of flocculus parameters ($G_{FL_{vestib}}$ and $G_{FL_{ecopy}}$) in low and high VOR gain states back to those in normal gain while other parameters were held at the values after adaptation. In this way, we could evaluate the system with the intact network configuration (cf. flocculocyst in the following text). The adapted low gain 0.42 becomes 0.30 if the floccular parameters are not plastic. In the case of high gain, the adapted gain 1.61 becomes 4.11. In either case, the elimination of the plasticity in flocculus does not return the adapted gains back to normal, rather it results in “excesses” of adaptation (Table 3).4

NO PLASTIC CHANGE BETWEEN PURKINJE CELLS AND FTNS. The roles of the plasticity currently identified between Purkinje cells and FTN were evaluated by the same method in the preceding text. Namely, the parameter $G_{PFTN}$ was kept at its value for normal gain state while other parameters were set to their values for low- or high-gain state. The result showed that if the signal transmission efficacy between Purkinje cell and FTN is not plastic, the adapted low gain becomes 0.65, and the adapted high gain, 1.41 (Table 3). This synthetic change appeared to be responsible for 56.1% (100% is 0.83–0.42 = 0.41) of learned low gain and for 25.6% (100% is 1.61–0.83 = 0.78) of learned high gain. However, one should note that this plasticity alone cannot yield these gain changes. It must work in concert with the other plastic sites in a particular network structure (see following text).5

INACTIVATION OF FLOCCULUS (FLOCCULECTOMY). The simulated VOR gains after flocculectomy for normal, low- and high-gain animals are summarized in Table 3. Although model parameters related to flocculus Purkinje cells that were estimated from experimental data changed significantly after low- and high-gain adaptation, learned VOR gains are not completely abolished after flocculectomy. Thus the conventional interpretation of flocculocyst can be misleading.6

Supplementary note 1. An inverse dynamic approach

Because Y neurons are one synapse removed from the motoneurons, a possible interpretation of the Y neuron discharges during OKS is that all the firing in Y neurons is translated into a motor command signal and could be accounted for using an inverse dynamic representation of the eye movement (Shidara et al. 1993; Takemura et al. 2001). This method of modeling the Y neuron discharges can be also justified because ocular pursuit is severely affected by lesions in Y neuron and completely abolished by removal of the floccular complex (Rambold et al. 2002). Thus we examine possible changes in eye parameters after VOR adaptation using this alternative analysis method (see Eq. 4). The major disadvantage of using this method is that retinal slip signal is unaccounted for, and therefore eye- and head-velocity values will be wrongly estimated. We nevertheless consider this method necessary to compare our results with those of other laboratories. Finally, to properly compare the results in Y neurons with those in Purkinje cells we have applied a similar inverse dynamic model (no retinal slip included) to extract the eye and head parameters of our previous Purkinje cell data (Blazquez et al. 2003).

The model will indicate the amplitude of the signal at the node, whereas red text next to each scope indicates the corresponding value from experiment.

Methods

We fit the neuronal response using a multiple linear regression model that includes eye, and head, but not retinal slip information. This method is based on an interpretation of the Y neuron response as an inverse dynamic representation of the eye movement and a feed-forward system that considers only vestibular information. This interpretation is not as anatomically plausible as the previous one, but it was used to allow comparison of our present results with other reports that did not use a retinal slip component in their analysis (Takemura et al. 2001). These reports analyzed the signal content of the neuronal discharge from their average response over several cycles of stimulation as a signal containing eye position, velocity, and acceleration during ocular following (no retinal slip term). Hence, in this third method, we averaged over cycles of stimulation. However, to explain VORs as well as OKR, our equation should contain also variables referring to the head-velocity and acceleration information to explain the Y neuron response during VORd (Lisberger et al. 1994b). The goodness of fit was quantified by means of the coefficient of determination (CD) (Takemura et al. 2001). The equations for this third method are

\[
f(t) = \frac{dx(t + \tau_f)}{dt} + \beta_0 \frac{dx(t) + \tau_f}{dt} + \gamma_x x(t + \tau_f) + \alpha_0 \frac{dh(t + \tau_f)}{dt} + \beta_h \frac{dh(t)}{dt} + \delta \]  

in Eq. 5, \( f(t) \), \( \hat{f}(t) \), and \( \bar{f}(t) \) represent the estimated firing rate, the observed firing rate, and the mean firing rate, respectively. Note that there are two major differences between the second and third analysis methods: the second analysis method uses retinal slip information to explain the neuron’s firing rate, whereas the third one does not, and the second analysis method uses the raw data to perform the multiple linear regression while the third uses the data resulting from averaging over cycles.

Description of the results

Changes in the Purkinje cell and Y neuron sensitivities after VOR adaptation. Floccular Purkinje cells change their eye- and head-velocity sensitivity in a nonmonotonic way and their eye-position sensitivity in a monotonic way (Supplementary Figs. 1 and 2). Hence this analysis method yields similar results to those reported by Blazquez et al. (2003). Y neurons, on the contrary, do not change their eye and head parameters significantly with the exception of head acceleration after low-gain adaptation (Supplementary Fig. 3).

Comparison of changes in the eye-velocity sensitivity of Y neurons and Purkinje cells after VOR adaptation (also see DISCUSSION). As explained in the preceding text, because the changes in Y neuron and Purkinje cells do not follow the same trend, we propose that a plastic site is located in the synapses Purkinje cell–Y neuron or as suggested by others (Nelson et al. 2003) that motor learning causes a change in the excitability of FTNs. Thus a direct comparison of the eye-velocity sensitivity of Purkinje cell and Y neurons suggest that the signal transmission between Purkinje cell–Y neuron (\( x \) in Fig. 4A) decrease 44% after low-gain training and 26% after high-gain training. In summary, using either an inverse dynamic model to explain Purkinje cell and Y neuron discharge or using a model that incorporates retinal slip information (see supplementary Fig. 4), our data suggest large changes in the signal transmission between Purkinje cell–Y neurons after low-gain VOR motor learning, and smaller changes after high-gain motor learning. The changes estimated after high-gain adaptation are less reliable than those after low gain because neither Purkinje cell nor Y neurons change their eye-velocity sensitivity significantly after high-gain adaptation.
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